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16. ABSTRACT

Fathead minnows (Pimephales promelas) were exposed during a partial chronic toxicity test to two DDT concentrations in the water, one in the diet, and combinations of water and diet for 266 days through a reproductive period of their life cycle. Tissue residue analyses were performed at preset intervals throughout the exposure and also on embryos, larvae at hatch, and 30- and 60-day progeny. The contribution of of DDT from each source was monitored with gas chromatography and liquid-scintillation techniques. The fish were fed a diet of clams that had accumulated ^{14}C-DDT when exposed at a DDT water concentration similar to that in the high fish exposure. Higher total DDT tissue residues were accumulated from the water than diet. Residues contributed by dietary DDT were additive to those from the water. Mean concentration factors were 1.2 for the diet and 100,000 for the water. Fish exposed to DDT in both water and diet had higher mortality rates than those exposed to only one or the other of these sources. DDT in the diet significantly reduced the probability of survival of the test fish ($P = 0.025$). Estimated maximum acceptable toxicant concentrations for DDT are 0.9 µg/l for fish exposed to DDT in the water only and 0.4 µg/l for fish exposed to DDT in both water and diet. Embryo residue levels and larval mortality rates were about twice as great for those from parent fish that had been exposed to DDT in both the water and diet as for those from parent fish exposed to only DDT in the water.

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DDT*	Carbon 14	Chlorinated hydrocarbons	6F
Bioassay*	Insecticides	Bioaccumulation	7C
Toxicity*	Food chain	Tissue residues	7E
Minnows			
Clams			
Aquatic animals			

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Long-Term Toxic Effects of DDT Food and Water Exposure on Fathead Minnows (*Pimephales promelas*)

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Fathead minnows (*Pimephales promelas*) were exposed during a partial chronic toxicity test to two DDT concentrations in the water, one in the diet, and combinations of water and diet for 266 days through a reproductive period of their life cycle. Tissue residue analyses were performed at preset intervals throughout the exposure and also on embryos, larvae at hatch, and 30- and 60-day progeny. The contribution of DDT from each source was monitored with gas chromatography and liquid-scintillation techniques. The fish were fed a diet of clams that had accumulated ^{14}C-DDT when exposed at a DDT water concentration similar to that in the high fish exposure. Higher total DDT tissue residues were accumulated from the water than diet. Residues contributed by dietary DDT were additive to those from the water. Mean concentration factors were 1.2 for the diet and 100,000 for the water. Fish exposed to DDT in both water and diet had higher mortality rates than those exposed to only one or the other of these sources. DDT in the diet significantly reduced the probability of survival of the test fish ($P = 0.025$). Estimated maximum acceptable toxicant concentrations for DDT are 0.9 μg/ℓ for fish exposed to DDT in the water only and 0.4 μg/ℓ for fish exposed to DDT in both water and diet. Embryo residue levels and larval mortality rates were about twice as great for those from parent fish that had been exposed to DDT in both the water and diet as for those from parent fish exposed to only DDT in the water.

Key words: toxicity, DDT, pesticides, food chain, ^{14}carbon, bioassay, minnows, clams, aquatic animals

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Lors d'un essai de toxicité chronique partielle, nous avons exposé des têtes-de-boule (*Pimephales promelas*) à deux concentrations de DDT dans l'eau, une dans la nourriture et dans des combinaisons d'eau et de nourriture durant 266 jours comprenant la période reproductrice de leur cycle biologique. Nous avons effectué des analyses de résidus dans les tissus à intervalles prédéterminés durant toute l'exposition, de même que sur des embryons, des larves à l'éclosion et des alevins de 30 et 60 jours. Nous avons suivi la contribution de chaque source en DDT par chromatographie en phase gazeuse et techniques de scintillation liquide. On a donné aux poissons un régime de coques qui avaient accumulé de ^{14}C-DDT lors d'une exposition à une concentration de DDT dans l'eau semblable à celle utilisées dans la haute exposition des poissons. Il y a plus grande accumulation totale de résidus tissulaires de DDT en provenance de l'eau qu'en provenance de la nourriture. Les résidus provenant du DDT alimentaire s'additionnent à ceux de l'eau. Les indices de concentration moyenne sont de 1.2 pour la nourriture et de 100 000 pour l'eau. Les poissons exposés au DDT à la fois dans l'eau et dans la nourriture ont des taux de mortalité plus élevés que ceux exposés à l'une ou l'autre de ces sources. Le DDT dans la nourriture diminue de façon significative la probabilité de survie des poissons d'essais ($P = 0.025$). Les concentrations acceptables maximales estimées de DDT sont de 0.9 μg/ℓ pour des poissons exposés au DDT dans l'eau seulement ou de 0.4 μg/ℓ pour des poissons exposés au DDT à la fois dans l'eau et dans le régime. Les niveaux de résidus dans les embryons et les taux de mortalité des larves sont environ du double chez ceux provenant de parents qui avaient été exposés au DDT à la fois dans l'eau et dans la nourriture comparativement à ceux dont les parents avaient été exposés au DDT dans l'eau seulement.

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THERE are many opinions among aquatic researchers about the importance of water concentration and food chain as sources for biological magnification of pesticides in the aquatic environment. Some authors suggest the food chain as the major source (Macek and Korn 1970); Harrison et al. 1970; Johnson et al. 1971; Eberhardt et al. 1971), whereas others (Reinert 1967, 1970; Chadwick and Brocksen 1969; Grzenda et al. 1970; Murphy 1971; Hamelink et al. 1971; Epifanio 1973) stress the water concentration. No data are available regarding those situations where both sources are involved at threshold levels of chronic toxicity.

Current pesticide standards are based upon water concentrations alone. We must also know what effect the presence of pesticide-laden food-chain organisms has on aquatic life so that accurate pesticide standards can be developed. The following study was initiated in response to these problems. Our objectives were to determine whether DDT accumulation in fathead minnows (*Pimephales promelas*) is more affected by a food or water source, to determine whether persistent pesticide exposure through both food and water is more toxic (or creates higher residues) than exposure through only one or the other of these routes, to estimate a partial chronic maximum acceptable toxicant concentration for DDT, and to determine DDT concentration factors for fathead minows and freshwater clams used as the food.

Materials and Methods

PHYSICAL CONDITIONS

All fathead minnow and clam exposures were conducted at the Environmental Research Laboratory-Duluth, Duluth, Minnesota.

Fathead minnow exposure — The basic design of the partial chronic exposure followed the recommended procedures set forth by the National Water Quality Laboratory Committee on Aquatic Bioassays (Jarvinen et al. 1976). A proportional diluter (Mount and Brungs 1967) was modified to deliver two test concentrations and a control with the low test concentration one quarter that of the high concentration. The toxicant was introduced by a 50-ml injector syringe with a Teflon® needle from an acetone stock solution. The highest level of acetone ever reached, within any 24-h period, in the high concentration test chamber was 5 mg/ℓ. Nominal DDT test concentrations, selected on the basis of acute and preliminary partial chronic data, were 2.0 and 0.5 μg/ℓ, respectively.

The test water was sand-filtered Lake Superior water sterilized with ultraviolet light and warmed to approximately 25°C by a coiled stainless steel heat exchanger located in a stainless steel headbox. A thermoregulator relay system (Syrett and Dawson 1972) activated tandem solenoid valves that controlled the flow of hot water through the heat exchanger.

The test chambers used for adult exposures measured 91 × 30 × 30 cm and held a water volume of 55 ℓ. Approximately 3 mo after the start of the test the adult tank was separated into two sections by stainless steel screen. Two 30.5 × 13.5 × 31.5-cm larval chambers were placed in the back section of each adult chamber, and flow rates were adjusted to provide 250 ml of test water to each larval chamber and 500 ml to the adult section. The flow of water to the test chambers was adjusted to maintain dissolved oxygen levels at >65% saturation and to provide a 99% replacement time of the test water in each test chamber within 10 h as determined from Sprague (1969).

Chambers were siphoned daily 1–2 h after feeding to remove leftover food and were brushed and siphoned weekly.

The photoperiod followed the normal daylight hours of Evansville, Indiana, except that it was necessary to extend the peak photoperiod of 15 h 45 min for approximately 6 wk to insure enough larvae for gas-chromatographic and liquid-scintillation analyses of tissue residues. Daytime light intensity varied from 269 to 441 lx in the adult chambers.

Clam exposure — Clams were exposed to ^{14}C-DDT in a flow-through system as described by Jarvinen et al. 1976. The toxicant was introduced into the toxicant chamber by syringe for a nominal DDT concentration of 2.0 μg/ℓ.

BIOLOGICAL CONDITIONS

Fish — On September 13, 1972, one hundred 45(±3)-day-old fathead minnows were randomly put in each of 12 test chambers. The 12 chambers were used to expose the fish through a reproductive period of their life cycle to duplicates of (1) a control, (2) DDT-exposed food, but no DDT in the water, (3) unexposed food, but 0.5 μg/ℓ DDT water exposure, (4) DDT-exposed food and 0.5 μg/ℓ DDT water exposure, (5) unexposed food, but 2.0 μg/ℓ DDT water exposure, and (6) DDT-exposed food and 2.0 μg/ℓ DDT water exposure. The fathead minnows were fed chopped and oven-dried clam tissue that was either clean or contaminated by ^{14}C-DDT.

Fish were sampled for total body tissue residues of DDT and percent lipids at 7, 14, 28, 56, 112, 224, and terminally at 266 days' exposure. The fish were not fed for 24 h before samples were taken. Ten fish were sampled from each test chamber after 7 days of exposure and five fish were sampled from each chamber thereafter, until the end of the exposure period when all remaining fish except those used for an elimination study were sampled. Samples were placed in preweighed glass vials, reweighed, and frozen at −8°C until analyzed. Results were determined on a whole-body wet-weight and lipid basis.

During the spawning period embryos in excess of the 50 required for hatchability studies were placed

in preweighed glass vials, reweighed, preserved with petroleum ether to prevent dehydration, and stored in a freezer at −8°C. Individual test-chamber samples were composited to provide a minimum of four 0.3-g (about 300 embryos) samples for residue analysis.

Larvae at hatch were transferred in groups of 40 to larval chambers for 30- and 60-day growth, mortality, and residue studies. The fish were photographed at 30 and 60 days by the method of Martin (1967) as modified by McKim and Benoit (1971) for growth determination. Tissue residues were analysed for two 30- and one 60-day samples for each of the 12 test chambers.

Larvae at hatch not saved for 30- and 60-day studies were weighed and grouped by the same method used for embryo-residue samples to provide a minimum of two 0.3-g (about 600 larvae) samples per test chamber for residue analysis. Residue analysis of larvae at hatch and embryos was performed on a whole-body wet-weight basis.

To simplify the presentation of results test treatments are coded as follows:

DDT exposure	Coding
Clean water, clean food (Control)	C
Clean water, DDT food	F
0.5 μg/ℓ DDT water, clean food	0.5 W
0.5 μg/ℓ DDT water, DDT food	0.5 W + F
2.0 μg/ℓ DDT water, clean food	2.0 W
2.0 μg/ℓ DDT water, DDT food	2.0 W + F

Results from duplicate chambers were combined, and the data are expressed as the mean ± SE unless otherwise indicated.

Clams — Chopped, oven-dried clam tissue was used as the food source. Five species of clams were collected from the Eau Claire River in Wisconsin: *Lampsilis siliquoidea, L. ventricosa, Lasmigona costata, Fusconaia flava,* and *Ligumia recta.* Before clams were exposed to DDT they were held for a minimum of 8 wk in a fiberglass tank through which lake water flowed. Each of four groups of clams was exposed to ^{14}C-DDT for 8 wk. The exposure time was chosen because preliminary studies indicated that an 8-wk exposure was necessary for the clams to achieve an equilibrium with an exposure concentration of 2.0 μg/ℓ DDT (the same DDT concentration as in the higher fish water exposure).

Clams were placed in the exposure chambers and slowly acclimated to 20°C. Fifty clams were used in each duplicate chamber per exposure, and 100 were held in a fiberglass control chamber through which lake water flowed from the same headbox as the exposure system. The control chamber had a volume of 170 ℓ and a water flow rate to provide 99% replacement in 6 h. Dissolved oxygen concentrations never dropped below 80% saturation.

The clams were fed daily with a commercial fish fry food and plankton. The flow-through apparatus was monitored daily, the chambers were siphoned every other day, and the sides of the chambers were scrubbed whenever algal or fungal growth became excessive.

After completion of an 8-wk exposure, the soft parts of the clams were removed, chopped in a blender, and oven-dried for 1½–2 h at 100°C. The dried clam meat was supplemented with a vitamin and mineral mix. A list of ingredients for the mix was obtained from the Fish-Pesticide Research Laboratory, Columbia, Missouri (Paul Mehrle, Fish-Pesticide Research Laboratory, Columbia, Missouri 65201, personal communication). The prepared food supply was kept frozen, and a small portion was removed daily for feeding.

Chemical Conditions

Fish — Water temperatures were maintained between 24.0 and 25.5°C and were checked daily in all test chambers. Routine water chemistries were determined weekly by the methods described by APHA et al. (1971). Dissolved oxygen levels were never lower than 5.4 mg/ℓ nor higher than 8.2 mg/ℓ. Mean total hardness, acidity, and alkalinity were 43.9, 2.8, and 42.5 mg/ℓ, respectively; pH was between 7.2 and 7.8. DDT stock solutions were prepared with DDT, Technical grade (*p,p'*-isomer 77%, Nutritional Biochemicals Corporation, Cleveland, Ohio) and DDT concentrations in the water were measured once a week. In each sample set, analyses were made on a duplicate and a spiked sample of control water. DDT was extracted from the test water with petroleum ether and analyzed by gas chromatography. Percent recovery from the spiked control water samples ranged from 38 to 115%; mean recovery was 86.9 ± 3.4% ($n = 39$).

Determined DDT water concentrations in the test exposures are presented in Table 1.

Clams — The *p,p'* DDT ring-UL-^{14}C (Mallinckrodt/Nuclear, St. Louis, Missouri) in benzene with a specific activity of 3.85 mCi/mM was procured in 50-μCi lots. The benzene was evaporated under a stream of nitrogen, and the *p,p'* DDT ring-UL-^{14}C was redissolved in 50 ml of acetone. The ^{14}C-DDT stock solutions were prepared by dissolving a calculated gram weight of DDT (Tech.) in acetone to which 45 ml

Table 1. DDT water concentrations in exposure chambers. SD in parentheses.

Nominal DDT water concn (μg/ℓ)	Measured concn (μg/ℓ)		
	N	Mean	Range
2.0 W	41	1.53 (0.35)	0.82[a]–2.30
2.0 W + F[b]	41	1.48 (0.30)	0.82[a]–2.00
0.5 W	41	0.35 (0.11)	0.16[a]–0.70
0.5 W + F[b]	41	0.37 (0.11)	0.19[a]–0.79
F[b]	41	0.01 (0.03)[c]	0.00 –0.10[c]
C	41	0.00 (0.01)	0.00 –0.06[d]

[a]Syringe injector malfunction (leakage).
[b]Fed clam tissue with ^{14}C-DDT (45.6 mg/kg).
[c]Leaching from ^{14}C-DDT food.
[d]Possible contamination from wrong food; occurred only once during exposure.

of the ^{14}C-labeled DDT in acetone was added. Total volume was brought to 250 ml with acetone and thoroughly mixed. Only enough ^{14}C-labeled DDT to give readable scintillation counts was combined with the unlabeled DDT. This procedure insured that the same stock of unlabeled material was the major source of DDT used in all fish and clam exposures. An additional benefit was a reduction in the amount of labeled DDT that would have been needed in the flow-through exposures. The clams were exposed to 2.0 $\mu g/\ell$ ^{14}C-DDT nominal concentration. The ^{14}C-DDT water concentrations ranged from 1.05 to 2.60 $\mu g/\ell$, with a mean concentration of 1.81 ± 0.13 ($n = 14$). Percent recovery of spiked control water samples ranged from 80 to 121%; mean recovery was 102.6 ± 8.5% ($n = 5$). Water analysis for DDT was performed by the same method as for the fathead minnow exposure.

Residue Analysis

Gas chromatography — An Eberbach Semi-Micro or Micro Container explosion-proof blender (depending upon sample weight) was used for extraction of adult fish and 30- and 60-day progeny. Anhydrous Na_2SO_4 was added to dry the tissues and insure homogenization. Samples were blended with petroleum ether for 2 min at high speed. The solvent extract was decanted onto an anhydrous Na_2SO_4 column and collected in a tared beaker for lipid determination. Each sample was extracted 3 times. The solvents were evaporated, and lipid weight was determined. The lipids were redissolved in petroleum ether and then cleaned up on a 20-g Florisil column. Samples were eluted with 200 ml of 6% ethyl ether–petroleum ether as described by the U.S. Department of Health, Education, and Welfare (1971). Samples were concentrated to a volume of < 10 ml on a steam bath. Analysis was completed by gas chromatography. Peak heights were measured individually for DDT, DDE, and TDE. DDT and metabolites were then summed to obtain the total DDT present. DDT, DDE, and TDE are expressed as the sum of the *o,p'*- and *p,p'*-fractions found in the analyses.

Samples of embryos and larvae at hatch were homogenized in 10 ml of petroleum ether in a glass tissue-grinding tube by using a Teflon pestle. Samples were extracted 5–8 times depending upon total sample weight and were concentrated as previously described for the adult fish. No cleanup was necessary and no lipid analysis was performed. Residue analysis was performed as for adult fish. Residue analysis on the clams was conducted on duplicate 1-g samples of tissue from both control and ^{14}C-DDT-exposed clams before addition of the vitamin–mineral supplement. The samples were extracted in a blender with 35% water–acetonitrile and then partitioned with 100 ml of petroleum ether. Cleanup was performed as described for the adult fish.

Liquid scintillation — We used a Packard Tri-Carb liquid scintillation spectrometer to determine DDT residues attributed to the food for all samples. Samples were prepared from the portion of the extracted tissue residues not used in gas-chromatographic analysis. The samples were evaporated to 0.2 ml in glass concentrator tubes and were then transferred to glass scintillation vials with four 1-ml washings of toluene. Fifteen millilitres of Instagel (Packard Instrument Company, Inc., Downers Grove, Illinois) (scintillation cocktail) were added to each vial. The vials were kept in the scintillation counter overnight to allow them to cool and dechemiluminesce before analysis.

A correlation was made between the gas chromatograms and scintillation counts through a series of dilutions of the ^{14}C-DDT stock solution. An average count per minute per microgram of DDT was then calculated and used for the determination of the micrograms of DDT attributed to the contaminated food source. Before final calculations were made, individual sample counts per minute were corrected for background radiation and sample volumes removed for gas-chromatographic analysis. Final results were calculated on a whole-body wet-weight basis.

Statistics

Mortality results were analyzed by calculation of the accumulative probability of fish survival over the different sample periods during the toxicity test as described by Jarvinen et al. (1976).

All survival and egg-hatchability data were transformed to arcsin $\sqrt{\%}$. Two-way analysis of variance was applied to all survival, embryo-hatchability, percent lipid, and 30- and 60-day progeny growth data to determine the effect of exposure to DDT in food or water. Dunnett's procedure (Steel and Torrie 1960) was used for comparison of treatment means with control means. We used nonlinear least square estimation (Dixon 1973) to determine values for the food and water parameters that affected adult survival.

Results

Adult Fish

Concentrations of total DDT (DDT + DDE + TDE) in the fish reached an equilibrium with the concentration in the diet within 28–56 days (Fig. 1). The additive residue effect of dietary total DDT when compared with residues from a water source only is also demonstrated (Fig. 1, 2). In general, total residues in fish exposed to DDT in food (F) and 0.5 $\mu g/\ell$ in water (0.5 W) peaked by 56 days, whereas concentrations in fish exposed to the other combinations of DDT in food and water peaked by 112 days. An equilibrium may have been reached at 0.5 W within 56 days. In general, residue levels decreased rapidly during the spawning period (112–224 days) and then increased after termination of spawning activity.

After 14 days of exposure, residues of DDT in fish exposed to DDT in both the water and

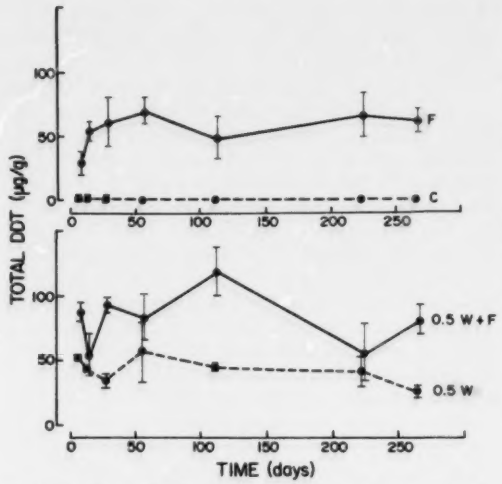

FIG. 1. Total DDT residues (micrograms per gram) in the controls and fish exposed to DDT in the food (*upper*) and in fish exposed to DDT in the water (0.5 $\mu g/\ell$) or in combination of food and water (*lower*). (Vertical lines indicate standard error.)

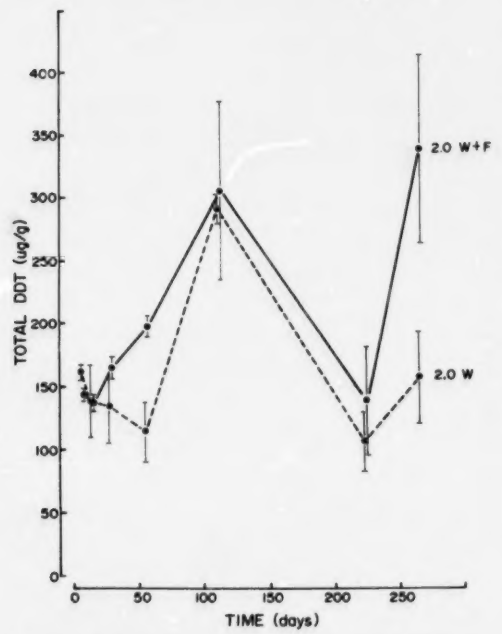

FIG. 2. Total DDT residues (micrograms per gram) in fish exposed to DDT in the water (2.0 $\mu g/\ell$) or in combination of food and water. (Vertical lines indicate standard error.)

food were generally greater than for those exposed to the insecticide in the water only.

DDE was the principal DDT residue found after 14 days of exposure, an indication that DDT was rapidly metabolized (Table 2). Lack of TDE in the residues of fish fed DDT-exposed clam tissue in the 7–56-day samples was caused by failure of the gas-chromatographic column to differentiate *p,p'*-TDE from *o,p'*-DDT. Column changes permitted differentiation for the 112 day and later samples. In general, DDT levels decreased rapidly after 14 days. The clams metabolized very little DDT to DDE; the principal metabolite was TDE (Table 3).

The ^{14}C-DDT content of the clam meat from the four exposures averaged 45.6 ± 3.8 $\mu g/g$ ($n = 4$) as determined by gas chromatography. This value indicates a concentration factor of 25,000 based upon the average concentration of DDT measured in the water. Total DDT in the clam tissue consisted of 68% DDT, 1% DDE, and 31% TDE.

Use of ^{14}C-labeled DDT in the contaminated food allowed us to use liquid-scintillation analysis (LS) to separate the DDT contributed by the food from the total amount of DDT as determined by gas-chromatographic analysis (GC). Gas-chromatographic and liquid-scintillation results were not identical as they should have been for the fish exposed to ^{14}C-DDT-contaminated food only. With $n = 19$, the mean liquid-scintillation value was 110% of the gas-chromatographic value with SD = 28.4%. To correct for this difference we adjusted LS values to GC values by the following formula: adjusted LS value = LS value obtained/ratio LS value to GC value for F-exposed fish. These data are presented in Table 4 and are expressed as a percentage of the total residues as determined by gas-chromatographic analysis. DDT in the food apparently had a maximum input within 28–56 days. The relative amount contributed by the DDT-contaminated food was about 60% of the total DDT residues in fish exposed at 0.5 W + F and about 30% in fish exposed at 2.0 W + F.

Mean calculated concentration of total DDT from food and water was 1.2 ± 0.1 times for the DDT from the food ($n = 57$) and 99,000 ± 7000 times for DDT from the water ($n = 39$, 0.5 W and 2.0 W exposures combined).

Lipid percentages were also determined for the test fish. No significant difference ($P = 0.05$) was found in the percentage of lipids between fish exposed to DDT in the water only and fish exposed to DDT in the water and fed DDT-contaminated food.

Fish fed a DDT-contaminated diet were less

TABLE 2. DDT, DDE, and TDE residues (micrograms per gram) in fish exposed to various test conditions (duplicate samples combined; each sample is a composite of 5–10 fish). SE is in parentheses.

Nominal DDT water concn (μg/ℓ)	Days of exposure																					
	7			14			28			56			112			224			266			
	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE	DDT	DDE	TDE
C	1.0 (0.5)	0.7 (0.2)	0.1 (0.1)	1.0 (0.2)	1.0 (0.0)	0.0	0.6 (0.1)	1.0 (0.5)	0.0	0.2 (0.0)	0.3 (0.1)	0.0	0.1 (0.0)	0.2 (0.0)	0.1 (0.0)	0.1 (0.0)	0.1 (0.0)	0.0	0.0	0.1 (0.2)	0.0
F	16.2 (5.7)	12.0 (3.4)	0.0	28.2 (3.2)	24.1 (4.0)	1.0 (0.1)	23.8 (4.7)	35.3 (12.9)	1.1 (1.1)	18.5 (1.5)	49.4 (8.6)	1.0 (0.2)	7.0 (1.9)	38.5 (13.9)	15.0 (3.8)	5.2 (1.7)	47.5 (11.7)	12.5 (3.8)	5.0 (1.0)	33.3 (4.6)	22.4 (3.9)
0.5 W	26.1 (0.6)	24.9 (0.8)	0.0	18.4 (5.4)	24.3 (4.7)	0.0	7.2 (2.0)	25.5 (2.9)	0.7 (0.0)	13.2 (6.8)	18.8 (7.4)	0.6 (0.6)	6.1 (0.3)	36.8 (1.0)	3.7 (0.7)	3.5 (1.0)	36.7 (11.4)	0.8 (0.2)	2.7 (0.7)	21.7 (3.2)	1.0 (0.2)
0.5 W + F	47.4 (5.4)	39.0 (7.7)	0.0	22.5 (10.6)	29.5 (5.2)	1.4 (0.5)	35.9 (7.4)	53.7 (2.0)	3.1 (0.6)	17.7 (3.4)	63.3 (14.4)	1.4 (0.5)	14.2 (5.1)	120.2 (33.8)	20.4 (5.2)	4.9 (2.1)	41.7 (17.2)	7.9 (3.7)	7.5 (1.5)	54.1 (7.0)	18.2 (2.9)
2.0 W	94.2 (5.3)	66.8 (0.9)	0.0	48.5 (8.8)	88.3 (19.6)	1.4 (0.3)	37.0 (13.9)	92.6 (14.1)	4.0 (0.9)	25.1 (8.2)	86.6 (14.8)	1.7 (0.3)	58.0 (0.6)	230.9 (34.9)	19.3 (1.6)	9.5 (2.5)	93.6 (20.7)	9.1 (1.0)	15.8 (4.6)	136.9 (31.4)	3.8 (0.8)
2.0 W + F	92.8 (4.9)	50.3 (0.0)	0.0	77.8 (23.2)	54.7 (28.3)	3.8 (0.8)	43.3 (3.7)	113.4 (12.1)	7.4 (0.2)	44.6 (0.5)	149.2 (8.4)	3.1 (0.9)	49.8 (19.0)	246.6 (67.8)	52.2 (13.5)	18.0 (7.3)	113.3 (32.8)	6.2 (3.3)	28.5 (8.1)	291.7 (62.0)	17.1 (5.1)

likely to survive than those fed clean food (Fig. 3). To test whether the addition of DDT-contaminated food altered the probability of survival, a two-way analysis of variance was run on the survival data. The hypothesis that the presence of DDT in the food does not change survival was rejected at the $P = 0.025$ level. The accumulative reduction in the probability of survival (Fig. 3) indicates two definite periods of high mortality, the juvenile stage during the first 28 days of exposure (test started with about 45-day-old fish; therefore, the fish were 73 days old at 28 days exposure) and during spawning between 112 and 224 days of exposure. Mortality was greater in fish fed DDT-contaminated food and remained high through 56 days' exposure. The death rate was also greater for fish fed DDT-contaminated food during the spawning period. Fish that died during the spawning period were predominantly adult males (about 83% males, 17% females). Residue levels in these fish were only about 55% of those in live sampled fish. Lipid percentages however, were very low (mean 1.07%, $n = 23$, all samples combined) when compared with the live fish (mean 3.1%, $n = 12$, all samples combined). The percentage of residue levels attributed to DDT in the food among fish that died

FIG. 3. Accumulative probability of survival for fish exposed to DDT.

TABLE 3. DDT, DDE, and TDE residues (micrograms per gram) in the fish food (oven-dried weight; 1½–2 h; 110°C). —, not detectable.

Exposures	Time period when used as food (days)	DDT	DDE	TDE	Time of year collected	Time of year exposed to ^{14}C-DDT
		^{14}C-DDT-exposed clams				
1	0–112	42.6	0.6	14.3	Fall	Spring (late)
2	112–224	38.2	0.5	2.7	Spring	Fall
3	112–224	31.2	0.2	9.7	Fall	Winter
4	224–266	11.0	0.2	29.0	Fall	Spring (early)
		Clean clams				
1	0–112	0.7	—	—	Fall	Spring (late)
2	112–224	—	—	—	Spring	Fall
3	112–224	—	—	—	Fall	Winter
4	224–266	—	—	—	Fall	Spring (early)

TABLE 4. Percentage of total DDT caused by the ^{14}C-DDT food source (duplicate samples combined; liquid-scintillation values shown are adjusted). SE in parentheses; *n = 1; **n = 4.

Nominal DDT water concn (μg/ℓ)	Days of exposure							Mean for entire period
	7 (n = 2)	14 (n = 2)	28 (n = 2)	56 (n = 2)	112 (n = 2)	244 (n = 4)	266 (n = 6)	
F	100.0 (1.4)	100.0*	100.0 (2.8)	100.0 (13.4)	100.0(6.2)	100.0 (2.7)	100.0 (3.0)	100.0 (1.6) (n = 19)
0.5 W + F	22.5 (0.0)	46.9 (19.4)	76.4 (3.4)	81.7 (4.2)	55.4 (1.4)	72.2 (5.4)	64.7 (1.4)	62.1 (4.2) (n = 20)
2.0 W + F	4.8 (2.1)	28.1 (15.7)	38.2 (3.4)	50.7 (5.6)	33.9 (0.9)	28.7 (2.3)	17.6** (1.2)	27.6 (3.4) (n = 18)

TABLE 5. Hatchability; mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in embryos from fish exposed to various test conditions (duplicate samples combined). N = number of spawnings; SE in parentheses; —, not detectable; LS = liquid scintillation.

Nominal DDT water concn (μg/ℓ)	N	% hatch	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	62	88.9	15	0.1 (0.0)	0.4 (0.0)	—	0.5 (0.0)	—
F	44	85.7	12	1.6 (0.1)	7.8 (0.9)	2.8 (0.2)	12.2 (1.1)	100 (6.2)
0.5 W	51	84.4	16	0.8 (0.1)	5.7 (0.6)	0.3 (0.0)	6.8 (0.7)	—
0.5 W + F	55	89.2	15	2.0 (0.4)	13.7 (2.2)	3.3 (0.6)	19.0 (3.1)	68 (1.8)
2.0 W	98	74.1[a]	23	3.8 (0.5)	19.6 (1.7)	0.6 (0.1)	24.0 (2.0)	—
2.0 W + F	51	78.3	18	5.3 (0.4)	31.3 (2.9)	4.2 (0.6)	40.8 (3.8)	28 (0.5)

[a]Significantly different from the control (P = 0.05). Dunnett's procedure (Steel and Torrie 1960).

during spawning was 100% of the DDT at the F exposure, 70% at the 0.5 W + F exposure, and 23% at the 2.0 W + F exposure. These percentages are similar to those found in the similarly exposed live fish.

EMBRYOS

Presence of DDT in the parents' food did not significantly alter hatchability, whereas DDT in the water did (Table 5). The reduction in hatchability, however, was significant only for embryos from parent fish that were subject to the 2.0 W exposure. The percentages of total DDT contributed by contaminated food fed to the parent fish are similar to those in residues from the adult fish. Residues from DDT-contaminated food ap-

TABLE 6. Mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in larvae at hatch from fish exposed to various test conditions (duplicate samples combined). SE in parentheses; —, not detectable; LS = liquid scintillation.

Nominal DDT water concn ($\mu g/\ell$)	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	6	—	0.2 (0.0)	—	0.2 (0.0)	—
F	4	3.0 (0.3)	18.6 (2.6)	4.8 (0.2)	26.4 (2.9)	100 (0.8)
0.5 W	4	3.8 (0.3)	18.9 (2.3)	1.3 (0.0)	24.0 (2.2)	—
0.5 W + F	6	5.7 (0.5)	32.8 (4.5)	5.0 (0.6)	43.5 (4.3)	60 (1.9)
2.0 W	9	19.9 (0.9)	66.5 (5.3)	1.5 (0.1)	87.9 (5.9)	—
2.0 W + F	5	20.1 (2.0)	73.0 (15.4)	2.9 (0.7)	96.0 (17.8)	20 (0.6)

pear to be additive to those attributed to water exposure.

DDE was the primary metabolite. Some DDT from the water was metabolized to TDE, but the amount is only about 10% of that attributed to the food source. There is increased TDE with higher water exposure for the embryos from the water plus food-exposed fish.

LARVAE AT HATCH

When compared with embryo residues, residues in larvae at hatch from parent fish fed dietary DDT are about 2 times higher, whereas those from parent fish exposed to DDT only in the water are about 3.6 times higher (Table 6). Part of this difference is explained by the reduction in weight of the larvae at hatch to almost one half that of the embryos. Therefore, if nearly all the residue were contained within the developing larvae, the calculated residue level would automatically be 2 times greater at hatch when the embryo membrane and surrounding fluid is lost. The percentage of total DDT resulting from DDT-contaminated food was about 8% lower for larvae at hatch than for embryos. Larvae were removed for analysis daily, so it is possible that some may have been exposed to DDT in the water for a maximum of 24 h before being removed.

DDE again is the principal constituent. High TDE levels in larvae from food-exposed parents are attributed to the food source.

PROGENCY AT 30 AND 60 DAYS

Residues of DDT in progeny are slightly higher at 60 than at 30 days, except for fish at the F exposure, where they are lower (Table 7). The percentage of total DDT contributed by the food source is only 8% higher at 60 days than that at 30 days. Progeny from fish exposed to 2.0 W hatched and raised in control water for 30 days contained only 0.5 $\mu g/g$ total DDT in their tissues. Progeny from fish exposed to 2.0 W + F, also hatched and raised in control water and fed clean food for 30 days, had residues of only 0.2 $\mu g/g$ total DDT. As determined by liquid-scintillation analysis, none of this DDT could be traced to the intake of DDT-contaminated food by parent fish. However, progeny from the same parent fish that were hatched and raised in clean water but fed DDT contaminated food for 30 days had total DDT residues of 31.6 $\mu g/g$, of which 93% could be attributed to the food.

In general, DDT residues decreased at most exposures between 30 and 60 days, whereas DDE residues increased. TDE residues also showed a slight increase between 30 and 60 days, except at the 0.5 W + F exposure, where there was a 38% increase.

DDT apparently did not affect the growth of the 30- and 60-day progeny. Two-way analysis of variance on growth data indicated that there was no significant growth difference ($P = 0.05$) between the progeny exposed to DDT in the food or water.

All larvae died within 5 days of hatch at the 2.0 W or 2.0 W + F exposures. Control larvae, in groups of 40 each ($n = 4$), transferred to these concentrations also died within 5 days. A significant effect ($P = 0.05$) on survival was observed for DDT water exposure at 30 and 60 days, but not for food exposure. Use of Dunnett's procedure (Steel and Torrie 1960) indicated that the exposures significantly different from the controls were those at 2.0 W and 2.0 W + F and those from parent fish exposed to 2.0 W + F, whose progeny were hatched and raised in control water and fed clean food for 30 days (Table 8). This latter group experienced a twofold higher death rate than progeny from parent fish exposed at 2.0 W that were hatched and raised in control water and fed clean food. In this higher mortality group, progeny in two groups (40 fish each) ex-

TABLE 7. Mean DDT, DDE, TDE, and total DDT residues (micrograms per gram); and percent total DDT contributed by the contaminated food in 30- and 60-day-old progeny of fish exposed to various test conditions (duplicate samples combined, progeny exposed to same conditions as parent fish unless otherwise indicated). SE in parentheses; —, not detectable; LS = liquid scintillation.

Parent fish nominal DDT water concn ($\mu g/\ell$)	Progeny exposure (days)	No. of samples	DDT	DDE	TDE	Total DDT	% total DDT caused by DDT in the food, LS adjusted
C	30	3	0.1 (0.0)	0.1 (0.0)	0.0 (0.0)	0.2 (0.0)	—
	60	3	0.1 (0.0)	0.2 (0.0)	—	0.3 (0.0)	—
F	30	4	8.1 (2.0)	15.4 (2.9)	12.2 (3.2)	35.7 (1.6)	100 (9.4)
	60	2	4.7 (0.4)	11.7 (0.7)	12.3 (5.8)	28.7 (5.7)	100 (4.1)
0.5 W	30	4	4.6 (0.9)	8.5 (0.9)	0.5 (0.2)	13.6 (1.8)	—
	60	5	6.1 (0.4)	17.3 (3.5)	0.6 (0.2)	24.0 (4.0)	—
0.5 W + F	30	4	12.5 (3.3)	18.6 (3.1)	15.3 (2.4)	46.4 (4.9)	60 (16.3)
	60	2	10.3 (1.0)	26.8 (0.5)	21.1 (0.9)	58.2 (2.4)	68 (0.4)
2.0 W[a]	30	3	0.2 (0.1)	0.2 (0.1)	0.2 (0.1)	0.6 (0.2)	—
2.0 W + F[a]	30	1	0.1	0.1	—	0.2	—
2.0 W + F[b]	30	1	3.2	9.3	19.1	31.6	93

[a]Progeny hatched and raised in control water and fed clean food.
[b]Progeny hatched and raised in control water and fed DDT food.

perienced zero survival and one group had 75% survival. Total DDT residue in the survivors was 0.18 $\mu g/g$. Another group of progeny from the same parent fish similarly hatched and raised in control water but fed DDT-contaminated food had only 19.5% survival with a residue level in survivors of 31.6 $\mu g/g$ total DDT.

Discussion

Our results indicate that uptake from the food is additive to the amount taken up from the water and that equilibrium with the food was reached within 56 days. These findings are similar to those observed by other authors. Grzenda et al. (1970) reported that there was no additional increase in body concentrations in goldfish after dietary DDT exposure (18 $\mu g/g$) for 32 days. Macek et al. (1970) reported an equilibrium with dietary DDT in the liver, brain, and skeletal muscle of rainbow trout after 28 days' exposure. The portion of the total DDT tissue residues in our test that could be directly attributed to the DDT-contaminated diet was about 30% in fish exposed to a concentration of DDT in water equal to that at which the food had been exposed and 60% in fish exposed to a water concentration one fourth that level. This appears to indicate that the main source of DDT uptake is the water. Chadwich and Brocksen (1969) exposed freshwater sculpins to dieldrin in diet and water and observed that a maximum of 16% of the dieldrin was accumulated from the food. They also stated that accumulation from the diet might be expected to be additive, but this was not so in their test. They reported residue levels that were not much different between fish exposed in food and water and in water only, whereas in our test, tissue residues between the two exposures were different after 14 days. Reinert (1967), also in a study with dieldrin, observed that only about one tenth as much of the residues in guppies was accumulated through ingestion of contaminated food when compared with residues in guppies exposed to a water concentration equal to that in which the food was exposed. This test, however, was of a shorter duration (32 days). In our study the highest residue level caused by DDT-contaminated food was one fourth that caused by exposure to DDT in water that was at the same concentration as that to which the food was exposed.

Our mean DDT concentration factor was 1.2 for dietary DDT and about 100,000 for DDT from the water. These values are similar to those observed by other authors. Hunt and Bischoff (1960) observed a concentration factor of 125,000 for brown bullheads from water, and Courtney and Reed (1972) observed a concentration factor of about 100,000 in tissues of golden shiners that were exposed to 0.3 $\mu g/\ell$ DDT in the water for 15 days. Reinert (1967) observed a 0.05 food concentration factor in tissue residues of guppies

TABLE 8. Percentage survival of 30- and 60-day-old progeny of fish exposed to various test conditions (larvae exposed to same DDT exposure as parent fish).

Progeny exposure (days)		Nominal DDT water concn ($\mu g/\ell$)			
		0	0.5	2.0[a]	2.0
		Clean food			
20	Tank A	73.8 ($n=4$)	55.0 ($n=4$)	25.0 ($n=2$)	0 ($n=3$)
	Tank B	55.8 ($n=3$)	65.6 ($n=4$)	62.3 ($n=2$)	0 ($n=3$)
	Grand mean	66.1	60.3	41.0	0[b]
60	Tank A	46.3 ($n=2$)	31.2 ($n=2$)	—[c]	0
	Tank B	45.0 ($n=1$)	66.3 ($n=2$)	—[c]	0
	Grand mean	45.8	49.0	—	0[b]
		DDT-contaminated food			
30	Tank A	73.0 ($n=3$)	57.5 ($n=3$)	23.6 ($n=3$)	0 ($n=2$)
	Tank B	69.9 ($n=3$)	36.7 ($n=3$)	0 ($n=1$)	0 ($n=2$)
	Grand mean	71.2	47.1	18.8[b]	0[b]
60	Tank A	62.5 ($n=1$)	35.0 ($n=1$)	—[c]	0
	Tank B	51.3 ($n=1$)	42.5 ($n=1$)	—[c]	0
	Grand mean	57.0	38.8	—	0[b]

[a]Progeny from parent fish were hatched and raised in clean water and fed clean food.

[b]Values significantly different from the control larvae (dupicate chambers combined), two-way analysis of variance and Dunnett's procedure (Steel and Torrie 1960); $P = 0.05$.

[c]No larvae available for 60-day study.

exposed to a diet of dieldrin-contaminated *Daphnia* (31 $\mu g/g$) for 32 days, but a concentration factor of 1.3 for *Daphnia* fed dieldrin-contaminated algae (71 $\mu g/g$). He stated, however, that the latter concentration factor may have been caused by algae ingested but not yet assimilated, as algal cells were observed in the *Daphnia* digestive tract. Epifano (1973) observed a concentration factor of 1.7 when he fed dieldrin-contaminated brine shrimp, *Artemia salina*, nauplii (0.213 $\mu g/g$) to crab larvae. Macek and Korn (1970) observed a food concentration factor of 0.6 for brook trout exposed to 3 $\mu g/g$ DDT in their diet for 120 days. Grzenda et al. (1970) estimated a total mean body residue level of 14.2 $\mu g/g$ DDT in goldfish after they were fed a diet containing 17.7 $\mu g/g$ DDT for 192 days, which would indicate a concentration factor of 0.8.

Some ^{14}C-DDT that leached from the food into the water was observed by liquid scintillation, and a mean ^{14}C-DDT water concentration of 0.065 ± 0.007 $\mu g/\ell$ ($n = 12$) was determined. If this level was bioaccumulated 100,000 times, the highest level that could occur in the fish would be 6.5 $\mu g/g$, or about 10% of the total food-contributed residue. However, the total DDT concentration measured in the water by gas-chromatographic analysis was not much greater when the fish were exposed to DDT-contaminated food only. If the control fish with a mean measured water concentration of 0.0029 $\mu g/\ell$ DDT are compared with fish exposed only to DDT in the food (F) with a mean measured DDT water concentration of 0.0123 $\mu g/\ell$, the difference is only 0.009 $\mu g/\ell$, a possible body residue of 0.9 $\mu g/g$ or about 1.5% of residues caused by DDT in the water leached from the food (assuming a 100,000 times magnification). Therefore, we believe that the contribution of labeled DDT through the water was negligible.

Fish exposed to DDT in the water exhibited higher total DDT residues at 7 days than at 14 days. This abnormal uptake pattern was caused by a decrease in lipid content of the fish which resulted from decreased food consumption. When the fish were first fed clam meat they would not feed; subsequently, a decrease of percent lipids resulted. When the fish began to feed normally, their lipid content stabilized and DDT tissue residues increased. Percent lipids changed similarly for the F exposed fish but since the only source of DDT exposure was the food, these fish showed a more typical DDT uptake.

Our results indicate that DDT in the tissues decreased between 14 and 266 days. During the same period DDE and TDE residues increased. Grzenda et al. (1970) observed similar results for goldfish fed a DDT-contaminated diet for up to 192 days. We also observed in our test that the TDE residues were high in fish exposed to dietary DDT and were low in fish fed clean food. This indicates that most of the TDE in the fish fed a contaminated diet probably came from the food itself, which was quite high in TDE.

The clams metabolized DDT almost entirely to TDE and produced little DDE. Some discrepancy was observed in the amounts of TDE produced between exposures. Although all clam DDT exposures were held at the same temperature (20°C), clams for exposures 1 and 4 were collected in the fall and exposed in the spring, whereas clams for exposures 2 and 3 were collected in the spring and fall and exposed in the fall and winter. More clam metabolism would naturally occur in the spring than in the winter, and a seasonally controlled mechanism might be responsible for metabolite differences among the DDT-exposed clams. Another possible explanation is that differ-

ences could have been caused by different ratios of clam species present. No check was made as to the relative frequency of each species, although the genus *Lampsilis* appeared to be most prevalent. The 1 μg/g DDT found in the control fish was probably caused by the presence of 0.7 μg/g DDT in the first batch of clean clam tissue fed to the fish. This explanation is likely since the use of this clam food and the residue peak in the control fish terminated at about the same time.

The proportion of TDE and DDE produced appears to vary with the type of organism exposed. Rats convert most DDT to TDE, whereas humans usually metabolize DDT to DDE (O'Brien 1967). Molluscs, as observed in this test and by Cooke and Pollard (1973), metabolize DDT mainly to TDE, whereas fish metabolize DDT essentially to DDE (Priester 1965; Johnson and Pecor 1969; Reinert and Bergman 1974). Ferguson et al. (1967), however, observed almost equal amounts of DDT, DDE, and TDE in several pooled samples of resistant mosquitofish (*Gambusia affinis*). Therefore, some fish can also readily produce large amounts of TDE.

Some breakdown of DDT to TDE may have occurred in our test when the clam tissue was oven-dried or when it was in frozen storage. Breakdown of DDT to TDE in frozen storage was demonstrated by French and Jefferies (1971). Our clam tissue, however, was used within 60 days, so postmortem breakdown is believed to have been negligible. Oven-drying also is not believed to have caused much DDT breakdown. Smith et al. (1973) found no large change in total TDE after fish steaks were baked at 177°C, and Metcalf (1955) states that pure DDT is stable up to 195°C.

Lipid values could be correlated with residue values, an indication that DDT uptake was influenced by the lipid content of the fish. DDT-residue levels declined rapidly during the spawning period. Lipid content of the fish also decreased at this time in fish exposed to the 2.0 μg/ℓ DDT water concentration whether they were fed clean or DDT-contaminated food. This is probably a fairly common occurrence. Reinert and Bergman (1974) observed that DDT residues were redistributed in the tissues of spawning-run fish and that this redistribution was closely related to a general decrease in the amount of fish oil.

The presence of DDT in the diet significantly reduced the probability of survival for exposed fish. Two definite periods of death were observed, the early larval stage up to 73 days of age and the spawning period, when mature males were the most sensitive. Fish that died during spawning were in relatively poor condition and did not feed. They probably used their fat reserves, thereby causing a release of stored DDT into the blood where the DDT could become toxic. Holden (1962) stated that fish in poor conditon or with low fat content were more susceptible to DDT toxicity. Our results support Holden's statement in that fish that died had predominantly lower lipid values than live fish sampled at the same time. Redistribution of DDT to the brain during weight loss with resultant deaths has been observed by some authors. Dale et al. (1962) observed high brain levels of DDT in rats that were starved after being fed DDT. Transfer of DDT to brains of birds and some resultant deaths were observed by Bernard (1966), Ecobichon and Saschenbrecker (1969), and Van Velzen et al. (1972). Redistribution of DDT and fat depletion in salmon and trout were observed during spawning run by Holden (1962) and Reinert and Bergman (1974) and in the laboratory in starved rainbow trout by Grant and Schoettger (1972). Desaiah et al. (1975) analyzed fish sampled at 56, 118, 225, and 266 days during our toxicity test and found that partial chronic exposure to DDT significantly inhibited mitochondrial Mg^{2+}-ATPase activity in the brain tissue of the fathead minnows.

In regard to toxicity in relation to the high concentrations of TDE in the food, the oral toxicity of TDE to rats (U.S. EPA 1972) is about one third that of DDE. In bluegills, however, the static acute data of Cope (1965) and Foster Mayer (Fish-Pesticide Research Laboratory, Columbia, Missouri 65201, personal communication) indicate that TDE is more toxic than DDE. Probably the relative toxicity of each varies with the specific organism exposed. Both DDE and TDE, however, appear to be less toxic than DDT (Oettingen and Sharpless 1946; Rudd and Genelly 1956; O'Brien 1967; Moyle and Skrypek 1969). Therefore, even though our clam tissue had high levels of TDE, our mortality results present a more accurate example of the DDT effect in a normal aquatic food chain than if we had used the more toxic unmetabolized DDT in a dry food mix.

To estimate the relative contribution of total DDT from the food to DDT from the water in regard to its effect on mortality the following model was assumed: the log of the total dose that a fish can tolerate has a normal distribution which can be approximated closely by a logistic distribution (Berkson 1951, 1953, 1955; Finney 1964). Thus, we have that

$$(1) \quad \ln(P/1-P) = \gamma + \beta \ln x$$

where x is the total dose delivered to the fish and

P is the probability of death given a dose at x. It was further assumed that DDT in water and food acts on the fish in a similar manner and thus x is proportional to the sum of water and food exposure and is expressed as

$$(2) \quad x = \theta\,(x_1 + \rho x_2)$$

where θ is a magnification and absorption factor in the target organism, x_1 = concentration of DDT in the water, $x_2 = 1$ if DDT is in the food, zero if otherwise, and ρ is the unknown amount of DDT ingested from the food. Substituting the value for x into equation (1) we have that

$$(3) \quad \ln(P/1-P) = \gamma + \beta\ln(\theta(x_1 + \rho x_2)) = \alpha + \beta\ln(x_1 + \rho x_2)$$

where $\alpha = \gamma + \beta\ln\theta$ is a combination of parameters that are estimable from the data. The probability of death, given a target dose of x, may be estimated from our data utilizing Abbott's formula (Finney 1971) from the relationship $Ps = (1 - Po)\,(1 - P)$, where Po is the probability of death of a control fish. Thus $P = 1 - Ps/(1 - Po)$ is an estimated probability of death due to DDT. Table 9 shows these estimated P values, the actual mean measured DDT water concentrations in duplicated exposure chambers, and the values of the estimated parameters obtained using nonlinear least square estimation. We used nonlinear estimation because ρ enters into the equation in a nonlinear manner, i.e. the amount of DDT ingested in the food and its effect on toxicity is unknown as compared to DDT sorbed from the water and this had to be estimated in the equation. The value $e^{-\alpha/\beta}$ is an estimate of LC50 caused by total exposure (Berkson 1944, 1953). It is estimated as 1.4596 μg/ℓ for DDT in water with no DDT in the food, or 0.9270 μg/ℓ for DDT in water with 45.6 μg/g DDT in the food. The amount of DDT in the food delivered to the target organism in this study is estimated as 0.5325 U in water. Thus, the percentage of DDT in the fish from DDT in the contaminated food in the 0.5 and 2.0 μg/ℓ DDT water exposures is estimated as 58.8 and 26.4, respectively. These estimates are very close to the measured percentages determined by liquid-scintillation analyses as shown in Table 4. The estimated relationship between probability of death and DDT in the water with and without DDT in the food indicates that at low DDT water concentrations the importance of the DDT-contaminated food is greater, and as the DDT water concentration increases the importance of the food effect decreases (Fig. 4). Dunnett's procedure (Steel and Torrie 1960) was used to compare the mean probability of the combined duplicate test exposure survival with that of the con-

TABLE 9. Estimated P values, average measured DDT water concentrations, and values for estimated parameters from nonlinear least square estimation for fish exposed to various test conditions.

Estimated P of death caused by DDT	$\ln(P/1-P)$	X_1 Avg measured DDT in the water (μg/ℓ)	X_2 (0 if no DDT in food) (1 if DDT in food)
0.0598	−2.7550	0.3517	0
0.4680	−0.1280	1.5285	0
0.1489	−1.7430	0.0123	1
0.1685	−1.5960	0.3740	1
0.7534	1.1170	1.4839	1

$$\ln(P/1-P) = \alpha + \beta\ln(X_1 + \rho X_2)$$

Parameters	Estimates of parameters	(SD)
α	−0.7661	(0.3802)
β	2.0261	(0.4313)
ρ	0.5325	(0.1946)

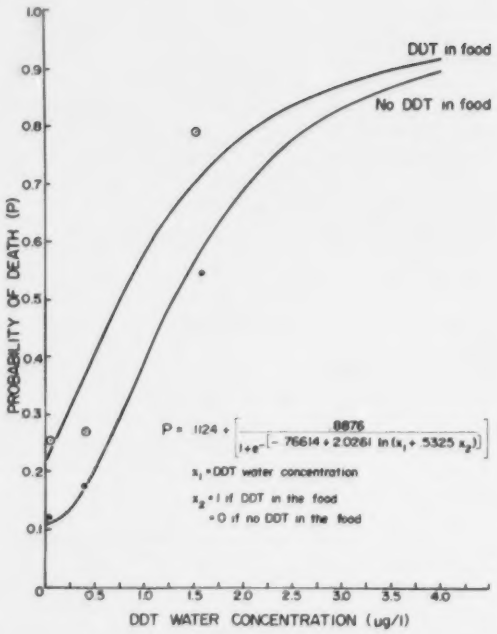
DDT in food
No DDT in food
PROBABILITY OF DEATH (P)
DDT WATER CONCENTRATION (ug/l)
P = 1124 + .8876 / (1+e^-[-.76614 + 2.0261 ln(x1 + .5325 x2)])
x1 = DDT water concentration
x2 = 1 if DDT in the food
= 0 if no DDT in the food

FIG. 4. Estimated probability of death for fish exposed to DDT at various water concentrations and fed clean or DDT-contaminated food.

trols. With $P = 0.05$, reduction in the probability of survival was significant only at the 2.0 W and 2.0 W + F exposures. By back calculation it is estimated that reduction in the probability of survival to 64.5% (35.5% mortality) is necessary to be significantly different. This mortality level would fall at about 0.425 $\mu g/\ell$ DDT in the water plus DDT in the food and at 0.925 $\mu g/\ell$ DDT in the water without DDT in the food (Fig. 4).

Our mortality data indicate that the presence of dietary DDT is more important when DDT water concentrations are low. A similar situation in nature could easily occur if food organisms built up tissue residues during pesticide contamination and then were eaten by predators that entered the area after pesticide water concentrations had moderated, or if several successive food-chain accumulations occurred. Dietary DDT exposure is important at relatively higher water concentrations, although to a lesser degree than water exposure. This view is also held by other investigators, such as Grzenda et al. (1970), who stated that apparent DDT biological magnification by the food chain may not be as significant as the length of time fish are exposed to pesticide residues in the water; Reinert and Bergman (1974), who stated that rapid accumulation of DDT residues in coho salmon was probably related in part to an increase in the intake of DDT-contaminated alewives; and Macek and Korn (1970), who demonstrated that dietary DDT can be very important, especially when compared with very low DDT water concentrations such as are found in Lake Michigan.

Embryo hatchability was reduced significantly only when parent fish were exposed to DDT at 2.0 $\mu g/\ell$ in the water and fed clean food. However, since DDT tissue residues were twice as high in embryos from fish exposed to DDT both in the diet and at 2.0 $\mu g/\ell$ in the water, the significant reduction of hatchability in the former group and not in the latter may have been the result of adult fathead minnow variability. Residues caused by dietary DDT were additive for all embryos from parent fish exposed to DDT in water and food. Huisman et al. (1971) reported that high residues of DDT, DDE, and TDE were accompanied by low fertility in pike. Kleinert and Degurse (1973) demonstrated a strong correlation between DDT content of embryos and larvae from walleyes in Wisconsin lakes, but could not associate the presence of DDT with success of the hatch. Burdick et al. (1972) demonstrated loss of brown trout and brook trout larvae hatched from eggs taken from female fish fed dietary DDT at different concentrations and durations of time. They also confirmed DDT as the cause of 100% mortality of larvae reared from eggs from lake trout fed 6 μg DDT/g body wt per wk. The average DDT residues in eggs less DDE were 7.61 and 11.92 $\mu g/g$ for 2 separate yr. In our study mean total DDT residues in embryos less DDE were 4.31 $\mu g/g$ from fish fed dietary DDT and 9.55 $\mu g/g$ from fish fed dietary DDT and also exposed to DDT in the water. Lack of 100% larval mortality at these levels is probably explained by species variability, as fathead minnows are generally less susceptible to DDT than salmonids. Larvae from fish exposed at 2.0 W and 2.0 W + F reared at the same water concentration as the parent fish experienced 100% mortality within 5 days. Part of this mortality may have been caused by DDT sorbed from the water, as larvae from these two parental groups transferred to control water for 30 days demonstrated an average of only 59% mortality among those from parents exposed to DDT in the water only and $> 81\%$ mortality among those from parent fish exposed to DDT in both the water and diet, an almost twofold higher mortality. These mortality data agree closely with embryo residue results that indicated almost twice as much DDT residue (40.9 vs. 24.0 $\mu g/g$) in embryos from parent fish exposed to DDT in both water and diet. Although residue levels were high, the larvae could either readily eliminate the DDT or dilute the residues through growth if placed in clean water. After 30 days in clean water these larvae, if they survived, had residues no greater than those in control larvae.

Total DDT residues were higher at all life stages whenever parent fish were exposed to DDT in both the water and diet than for water exposure alone. Even though the concentrations of DDT accumulated from water are higher than those accumulated from food, the presence of DDT in the diet caused higher tissue residues and death rates than were found in fish that were only exposed to DDT in the water. Death rates were not significant for larvae exposed to dietary DDT at 30 and 60 days, perhaps because some larvae could not adapt to clam tissue when active feeding commenced, and deaths occurred among the control fish by starvation. This was reflected in a mean mortality of 34% among control larvae at 30 days.

A "just safe" water concentration or maximum acceptable toxicant concentration (MATC) determined from mortality results would be about 0.9 $\mu g/\ell$ DDT for water exposure alone and about 0.4 $\mu g/\ell$ DDT with the added presence of dietary DDT. Application factors as defined by Mount and Stephan (1967), using a 96-h TL50 value of 48 $\mu g/\ell$ DDT demonstrated in one of

our acute fathead minnow toxicity tests and the maximum acceptable toxicant concentrations above, would be 0.0188 or 1/53 for fish exposed to DDT in the water alone and 0.0083 or 1/120 for fish exposed to DDT in both the water and diet. Consequently, food as well as water sources of exposure to certain materials must be considered when toxicity tests are designed or the conclusions drawn from such tests are evaluated.

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